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(5) INTRODUCTION:

Breast cancer is now recognised as an heterogeneous disease in which there are multiple molecular abnormalities which progressively accumulate to result in the clinical and morphological phenotypes seen as breast cancer. As the dissection of these molecular events is undertaken at the gene level it is essential that relevant cell systems are established to act as future targets in which to understand the function of the proteins encoded by these genes. In particular it will be necessary to establish human models in which to study the function of predisposing genes. Also it is essential that systems are established now that will enable researchers to study the importance of combinations of molecular genetic abnormalities and their relative contributions to the tumor phenotype. In parallel it is important that material is available from the earliest stages of malignancy which can be used to assess the relevance of the *in vitro* and molecular data.

This project is in two parts, which is focussed on developing an infrastructure resource that will enable research groups to address questions particularly related to the early stages of breast cancer evolution, and also to provide systems that will enable advances to be made related to prevention, diagnosis and treatment.

(I) Part 1 Familial Breast Cancer:

Between 5-10% of breast cancer is due to cancer predisposing genes. In the United Kingdom, there are 25,000 new cases of breast cancer per year, therefore about 2,500 cases each year could be due to a cancer predisposition gene. Two genetic models could account for the genetic predisposition to breast cancer. The first is the presence of a rare, but highly penetrant gene which would account for about 10% of all breast cancer cases; the second is a more common, less penetrant gene which would confer a lower cancer risk to each individual gene carrier, but due to its wider distribution, such a gene would contribute to a larger number of breast cancer cases (maybe as high as 86%). It is now clear that familial breast cancer is a heterogeneous disease, and a combination of these two models is the most likely.

An autosomal gene BRCA1 with high penetrance has been cloned (1) and gene carriers have a lifetime risk of breast cancer of 80%. The Breast Cancer Linkage Consortium has obtained linkage for another predisposing gene BRCA2 on chromosome 13q12-13 (2). Although rare, germline mutations in the p53 gene confer a very high breast cancer risk - 90% by age 50, (3). It is likely that lower penetrance genes contribute to a larger percentage of overall population breast cancer risk. One such candidate would be ataxia telangiectasia (AT) since AT heterozygotes have a relative risk of breast cancer at about six times that of the general population (4). The AT gene has been recently cloned (5).

This project is aimed at providing a resource of cells, cell lines and frozen tissues from patients that have an increased risk of developing breast cancer due to the fact that they are carriers of breast cancer susceptibility genes. Included in the study are patients from families with BRCA1, BRCA2, Li-Fraumeni and Li-Fraumeni syndromes and patients with ataxia telangiectasia. Establishing these cells in culture will provide systems for both primary studies of the abnormal genes in comparison with the wild type, but also models in which to study synergistic effects of genes, so enabling analyses of the early events in breast cancer.

Such *in vitro* systems will also provide relevant models to:

- a) explore the reversal of the predisposed phenotype using genetic manipulation;
- b) carry out drug testing for both prevention and treatment;
- c) test radiation sensitivity to enhanced risk.

In order to study the effects of putative breast cancer predisposing genes, it is necessary to have access to a bank of cells of an appropriate phenotype, derived from such individuals. As the great majority of breast cancers are derived from luminal cells in the breast epithelium, it is these cells that must be acquired and established *in vitro* as the primary resource. In addition, however, there is accumulating evidence for a role of fibroblasts in both the modulation of mammary morphogenesis and tumor progression. In order to cover all possible mechanisms of action of predisposing genes it is therefore necessary to establish cultures of stromal cells and myoepithelial cells from the same patients.

A number of groups including our own have, over the past decade, developed methods whereby the cells from human breast epithelium, which include both luminal and myoepithelial types, can be cultured *in vitro* and cloned (6). However, we are the first group to have developed methods whereby the constituent cells of this epithelium can be separated and cultured as pure cell populations. Our initial studies utilised FACS and exclusively expressed surface antigens present on the different epithelial cell types to sort them. This method has given populations of very high purity (>98%) but in relatively low yields (<10⁵ cells/preparation). Such preparations have, however, enabled us to demonstrate that it is the myoepithelial cells, which de-differentiate in culture to give a simple basal epithelial phenotype, which rapidly come to dominate 'mixed' cultures derived from the intact epithelium. As such cells do not seem frequently to give rise directly to breast cancers, they must be separated in bulk from the luminal component if relevant culture systems are to be established from genetically pre-disposed individuals. In addition, methods have had to be developed for efficient conditional immortalisation of small numbers of cells (described in more detail in Section 6).

We have been successful in obtaining breast tissue from a number of women with an increased familial risk of breast cancer and have established primary cultures from a high proportion of these.

(ii) Part 2 *In Situ* Data-Base:

Owing to the breast screening programmes, pathologists are seeing an increasing number of small tumors of which approximately 25% are ductal carcinoma *in situ*. This is providing more material for experimentation, but as these lesions have a good prognosis, long term follow up is required before any parameters measured in these tumors will be evaluable as predictors of behaviour. It is, therefore, essential that large series of retrospective cases are accumulated that can be used to correlate the results of future *in vitro* gene expression experiments with the *in vivo* pattern of expression and how it relates to the stage in tumor progression and parameters that can be measured directly in tumor samples. In addition it is clear that *in situ* breast cancer is itself an heterogeneous disease at the molecular level (7,8,9). To arrive at answers as to the relative importance of new genetic abnormalities it will, consequently, be necessary to combine the data from many large centres that specialise in breast cancer. It can be predicted that pathologists

will in the future be defining a molecular "bar-code" of *in situ* disease which will give predictive rather than prognostic information. It is, therefore, essential that large banks of early lesions are available to assess the relative importance of individual genetic abnormalities and the order in which they occur. By pooling material and data it will be possible to obviate the reporting of small series that are often misleading and remain unsubstantiated. In this context the objective of this part of the proposal is to identify a well characterized group of *in situ* cancers.

The Breast Diagnostic Unit of the Royal Marsden Hospital recruited its first patient in 1967. The objective was to offer a screening service to women who were perceived to have a high risk of breast cancer. The criteria used for defining a family history at that time were rather ill defined and thus all patients with a first or second degree relative affected were recorded. Clinical data, mammograms, and information on risk factors are recorded on the majority of the 30,000 patients seen since that time which includes over 600 cases with pure *in situ* carcinoma, according to the original pathology reports. The *in situ* cancers in this data set are a self selected population and thus not representative of a modern screening population; however, the material is valuable owing to the long median follow up and its use for molecular and immunohistochemical studies. A priority has been in this first year to establish a separate data-base of the *in situ* cases and to review the pathology using modern criteria. This has been done in conjunction with a record of all the clinical data available relating to the macroscopic appearance of the lesions, diagnostic tests, treatment and follow-up (see APPENDIX for information now included on the data-base). During the pathology review we have identified representative paraffin blocks that contain material for future studies.

(6) BODY

(i) Familial Breast Cancer In Vitro

a) The Structure of the Cancer Family Clinic

Risk estimates are computed from the family structure. If the family is likely to be carrying BRCA1 (families with both breast and ovarian cancer or families with >4 cases at less than 50 years), the risks are computed from the Breast Cancer Linkage Consortium data. Risk figures for Li-Fraumeni and Li-Fraumeni-like families are computed as per Garber *et al.*, 1991, where gene carriers have a 90% risk of breast cancer before 45 years. Risk figures for individuals in families unlikely to be due to BRCA1 or p53 are computed from the Claus study (10). Referrals are sent from surgeons, oncologists and mammography screening centres nationwide. We have had problems in receiving sterile specimens from other hospitals. This has resulted in the loss of many specimens due to bacterial or fungal contamination, presumably carried over from the pathology cut up area. However, in spite of these limitations we have managed to establish cultures from 20 referred cases and have commenced immortalisation of 6, using the methods described below.

a) Material resources:

Until the last few months the genes (other than mutant p53) which pre-dispose to breast cancer in a familial context had not been identified. Even though the BRCA1 gene has been identified the women in the UK coming to surgery do not have access to the test for mutations in this gene. This is likely to change in the next 6-9

months. We are currently using samples from normal individuals at high risk of subsequent cancer, in whom the likelihood of being a carrier is calculated at 25% or greater. These samples are used on the advice of the risk estimates assessed as above. This approach requires the processing of a relatively large number of samples so as to ensure that at least some samples are from *bona fide* carriers. These samples will then be tested retrospectively once the tests are routinely available. As can be seen from Table 1 in the Appendix we have received samples from 21 patients for this project, but in addition we have banked an additional 10 samples from normal cosmetic breast reduction samples. We intend in the future to collect epithelial cells and fibroblast preparations from all specimens coming to us from cosmetic operations (approx 30 additional cases/year).

b) Tissue preparation:

On receipt of the specimens they are subjected to routine pathology description and investigation for which Professor Gusterson is responsible. All patients at the Royal Marsden Hospital give informed consent for all tissue removed at operation to be used for research purposes.

Samples for culture are processed as described previously (6). Briefly the breast tissue is chopped into a fine mince with scissors and the epithelial "organoids" prepared by progressive collagenase digestion, sedimentation and filtration. Primary epithelial cultures will be prepared by seeding 1,000 to 2,000 stroma-free organoids into 75 cm² plastic culture flasks in RPMI 1640 medium with 10% (v/v) fetal calf serum, 5µg/ml hydrocortisone, 5µg/ml of insulin and 100 ng/ml of cholera toxin plus penicillin and streptomycin. After 7 days, when the organoids have mobilised and spread to form near-confluent epithelial cultures cells are harvested by trypsinisation. Samples of all cell types in primary epithelial cultures are harvested and stored, in replicate, as frozen cell samples in liquid nitrogen. These can be retrieved and used at a later date for bulk cell preparation using the methods described. In this first year, only the Li Fraumeni patients have had a proven genetic phenotype and thus in the majority of cases we have not processed the tissue further.

d) Epithelial cell separation and immortalization:

Mixed epithelial cultures have been further processed in selected cases by MACS sorting on the basis of the exclusive expression of the epithelial membrane antigen by luminal cells and the expression of CD10 on myoepithelial cells as previously described (11). Using this type of methodology it is possible to produce in excess of 10⁷ cells. Purified populations of cells have been obtained where possible and stored for further analyses.

The following explains the technique used to establish immortalized cells. Having established a high titre amphotropic packaging line producing replication-disabled retrovirus that encodes the tsA58-U19 gene within the pZip(neo)SV(X)1 vector, we have used this to immortalize purified human mammary cells in the following manner. FACS sorted preparations of epithelial membrane antigen positive cells have been established in short-term clonal culture, as described by (6). After selection for the neomycin resistance gene that forms part of the vector, a pure population of tsT-antigen expressing cells is obtained (9). Fibroblasts are also purified from the digested breast tissue and stored in liquid nitrogen.

Although an SV40 based system has limitations in so far as effects of the viral gene are concerned these are minimised by the use of a temperature sensitive system. At this time it is the most efficient and controllable system available for reproducible immortalization of human cells. As stated above we have commenced the immortalization process on cultures from 6 patients/women.

In the original proposal we set ourselves a number of tasks. Below is a summary of achievements measured against the objectives:

Objective:

Task 1, Separation and banking of epithelial and stromal cell types from breast tissue of predisposed individuals, Years 1-2:

- a. Breast tissue will be separated into component cell types using a combination of immunomagnetic (epithelial) and selective digestion (stromal) techniques.
- b. Cultures will be assessed for relevant purity using flow cytometry of cell-type specific antigens and multiple immunofluorescence methods.
- c. Pure cultures will be banked in replicate in liquid nitrogen to await identification of specific predisposing genotype.

Achievement:

We aimed to use years 1 and 2 to produce the primary cultures. In many preparations a,b and c have been achieved. We have had to train a new member of staff in this difficult technique so the success rate has been very good. In year 2 we aim to proceed with more specimens and to commence the immortalization of more selected cultures where we know the molecular phenotype. Professor Ponder in Cambridge and Professor Pierotti in Milan have kindly agreed to assist with the BRCA1 screening and the p53 mutations are being analysed in collaboration with Dr Eeles who is a co-applicant on the grant. The BRCA2 mutations will be analysed in collaboration with Dr Stratton once the gene has been cloned.

Task 2, Establishment of cell lines from specific genotypes, Years 2-3:

- a. Examples of high penetrance genotypes will be immortalized using retroviral gene transfer.
- b. Resulting lines will be characterised with respect to their growth and functional responses, compared with non-predisposed cases.

(li) Establish a data-base and tissue bank of *in situ* disease

The cell biological resource produced by the technique described above will facilitate research by producing cell systems that can be utilised for analyses of genes involved in the multistep process of breast cancer. It will, however, be necessary to constantly return to the actual disease to assess the relevance of these findings. It is therefore the purpose of this part of the proposal to establish a data-base of patients presenting with purely *in situ* breast cancers and epithelial atypias at the Royal Marsden Hospital since 1967.

We have identified over 600 cases recorded as *in situ* carcinoma that are to be considered for incorporation into the data set. In the Appendix a table of the information to be recorded on the data-base is provided together with examples of the Histopathology Review Form, the Patient Information Check List that has been used and an example of the patient data recorded. The following has been carried out and recorded on the 450 cases that have been reviewed so far and are on the data-base.

a) The histology of all material on these cases has been reviewed by Professor Gusterson and information put on the data base from the Histopathology Review Form. The review form is identical to that used in the UK National Screening Programme. This form has, however, been recently amended to incorporate a new definition of DCIS and its grading (12). The grading system is based on that agreed by the European Pathologists Working Group and in addition includes a definition of atypical ductal hyperplasia using the criteria of Page (13, 14).

b) Tissue blocks that contain sufficient material have been identified and marked for future study. In particular data has been recorded to identify interesting cases where transitions could be defined from normal, through epithelial proliferation without atypia, to atypia, and *in situ* carcinoma.

c) We have started to cut one unstained section and 10 unstained sections mounted on silane coated slides for future use. In addition, blocks have been identified that have sufficient material for microdissection of DNA from specific lesions.

d) In all cases clinical information has been recorded for future clinico-pathological correlations. In the next year patients not still being followed up by the Royal Marsden Hospital will be flagged, so that registration of subsequent cancer in the case of the benign diseases, and of death can be recorded. This will be done in conjunction with the National Health Service Central Register and the Local Cancer Registries.

e) Because patients coming to the Breast Diagnostic Unit were considered to be of high risk, they include many cases that appear to have a family history of breast cancer. It is, however, essential that proper family histories are taken. We have now carried out family histories on all cases of LCIS as part of another study, but the data will be incorporated into the data-base. Within this data set there are cases of metachronous and synchronous bilateral disease (See Appendix). These have been recorded. Family histories on the DCIS cases will be carried out in the next year.

f) We have identified those cases of *in situ* cancer where it is difficult to establish the presence or absence of microinvasion, as these may be useful for future studies.

We have therefore made a considerable impact on our objectives having almost completed the review and the data-base in year 1. The priority is to complete the data-base and to commence studies based on this material in addition to the objectives identified above. We aim to add all of the new cases from 1993-1995 and to screen the pathology records for borderline (atypical) lesions. It is clear that pathologists have great difficulty in agreeing an objective criteria for diagnosing

atypical lesions. It is to be predicted that the present data-base will provide definitive answers to recent interesting data on the expression of cyclin D mRNA as a distinguishing marker of malignant lesions (18). This observation is the first of many in this important area and a data-base of material will aid such investigations. Studies currently under investigation:

1. The use of the data-base to assess the best methods of grading of DCIS: This is based on a comparison of three proposed methods (13,14 and 15). It is intended to compare these methods using the long follow-up available on the data-base and to see if it is possible to identify those tumours that will have local recurrence following surgical excision. This is an important study as there is a need for a consensus on the classification to be adopted and it is hoped that this study may lead the way.

2. Professor David Page is coming as a visitor in February 1996 to read the slides and to ensure that the criteria for atypical ductal hyperplasia are correct in the data-base. In addition the cases are to be used for a proposed collaboration between our two groups on the role of the BRCA1 gene product in *in situ* breast cancer.

3. We are expecting to be able to use this material for molecular studies to address specific questions in relation to the variant phenotypes seen in *in situ* breast cancer using LOH analyses at specific loci. These studies will, in the first instance, concentrate on LCIS / ALH and the solid small cell variant of DCIS that appears to have a phenotypic overlap with LCIS. These studies are supported by other funding sources and will utilise the expertise that we already have in this area (19)

(7) CONCLUSIONS:

We have made significant progress in the last year in meeting our objectives and targets. In relation to the familial breast cancer work the major change that we have had to consider is the cloning of BRCA1. This has meant that we have decided to wait until we can screen for the mutations in the women that we have samples from before immortalization. To date we have therefore mainly concentrated on starting immortalization on the Li Fraumeni cases.

In relation to the *in situ* data-base: Progress has been faster than we predicted and we should have the data recorded on all available cases by August 1996. In addition we will have most of the sections cut by that time. We have had to make an assessment of which blocks are the best to use for staining and molecular studies. This is due to the fact that the review has shown that in many cases the diagnosis was based on a small focus of abnormal proliferation that is no longer in the block. This is important for any clinical correlations and indicates the problems of sampling bias that can be introduced into certain studies using this material, where clinical parameters are used as an end-point. For molecular correlates of morphology, however, the data is very valuable.

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(9) APPENDIX

TABLE 1

CASE	STATUS	FROZEN TISSUE	PRIMARY CULTURE	SEPARATED CELLS			
				LUMINAL	MYOS	FIBS	KERAT
1	LFL	N	Y	N	N	Y	Y
2	LFL	N	Y	N	N	Y	Y
3	LFL	N	Y	N	N	Y	Y
4	LFL	N	Y	N	N	N	Y
5	CONTROL	N	N	N	N	N	N
6	FH	Y	Y	N	N	Y	N
7	FH	Y	Y	N	N	N	N
8	Klinefelters	Y	Y	N	N	N	N
9	FH	N	Y	Y	Y	N	N
10	FH	N	N	Y	N	Y	N
11	FH	Y	Y	Y	Y	N	N
12	FH	Y	N	N	N	N	N
13	FH	Y	N	N	N	N	N
14	CONTROL	N	N	Y	Y	Y	N
15	FH	N	N	Y	Y	Y	N
16	CONTROL	N	N	N	N	Y	N
17	CONTROL	N	N	Y	Y	N	N
18	FH	Y	N	Y	Y	N	N
19	LFL	Y	Y	Y	Y	Y	N
20	LFL	Y	Y	Y	N	Y	Y
21	FH	N	Y	Y	Y	Y	Y
22	CONTROL	Y	N	Y	Y	Y	N
23	FH	N	N	Y	N	Y	N
24	FH	Y	Y	Y	N	N	N
25	FH	Y	N	Y	Y	Y	N
26	FH	Y	N	N	N	N	N
27	FH	Y	Y	N	N	Y	N

KEY: FM Family Members

LFL Li-Fraumeni

BREAST SCREENING HISTOPATHOLOGY

Surname Forenames Date of Birth

Screening no Hospital no Report no

Side ☐ Right ☐ Left Histological Calcification ☐ Absent ☐ Benign ☐ Malignant

Specimen radiograph seen? ☐ Yes ☐ No Mammographic abnormality present in specimen? ☐ Yes ☐ No ☐ Unsure

Specimen type ☐ Localisation biopsy ☐ Open biopsy ☐ Segmental excision ☐ Mastectomy ☐ Wide bore needle core

Specimen size (excluding mastectomies and needle core biopsies) x x mm.

HISTOLOGICAL DIAGNOSIS ☐ NORMAL ☐ BENIGN ☐ MALIGNANT

For BENIGN lesions please tick the lesions present

- ☐ Fibroadenoma ☐ 'Fibrocystic change'
- ☐ Papilloma ☐ Single ☐ Solitary cyst
- ☐ Multiple ☐ Periductal mastitis/duct ectasia
- ☐ Complex sclerosing lesion/radial scar ☐ Sclerosing adenosis
- ☐ Other (please specify)

EPITHELIAL PROLIFERATION

- ☐ Not present ☐ Present with atypia ('ductal')
- ☐ Present without atypia ☐ Present with atypia (lobular)

For MALIGNANT lesions please tick any of the following present

NON-INVASIVE

- ☐ Lobular ☐ Paget's disease ☐ Ductal ----- Subtype

MICROINVASION

- ☐ Not present ☐ Possible ☐ Present

- ☐ Cribriform
- ☐ Solid
- ☐ Papillary
- ☐ Micropapillary
- ☐ Comedo

INVASIVE

- ☐ 'Ductal' (Not otherwise specified) ☐ Tubular or cribriform carcinoma
- ☐ Medullary carcinoma ☐ Mucoid carcinoma
- ☐ Lobular carcinoma
- ☐ Other primary carcinoma (please specify)
- ☐ Other malignant tumour (please specify)

MAXIMUM DIAMETER (invasive component) mm (in-situ) mm

AXILLARY NODES

☐ Not Present Number positive Total Number

OTHER NODES Site

☐ Not Present Number positive Total Number

EXCISION

☐ Reaches Margin ☐ Does not reach margin (Distance mm) ☐ Uncertain

GRADE

☐ I ☐ II ☐ III ☐ Not assessable

DISEASE EXTENT

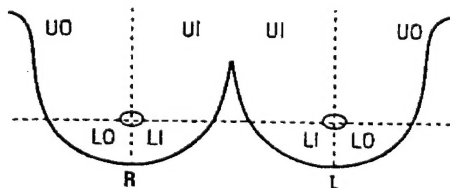
☐ Localised ☐ Diffuse single quadrant ☐ Multiquadrant ☐ Not assessable

VASCULAR INVASION

☐ Present ☐ Not seen

COMMENTS/ADDITIONAL INFORMATION

SITE (Optional)



PATHOLOGIST

☐ Case for review?

DATE

INFORMATION CHECKLIST
(Information search in patient's records etc.)

1	SURNAME	HOSPITAL NUMBER		
	DATE OF BIRTH			
2	ASYMPTOMATIC SYMPTOMATIC (tick)			
3	FAMILY HISTORY			
	BREAST CANCER	RELATIONSHIP	AGE AT DIAGNOSIS	
	OTHER CANCER	RELATIONSHIP	TYPE	AGE
4	HOSPITAL & DATE OF OPERATION	1	2	3
5	BREAST/SIDE			
6	SITE IN BREAST			
7	OPERATION PERFORMED			
8	CANCER OR BENIGN DISEASE TYPE			
9	SPECIMEN RADIOG./ ABNORM. PRESENT			
10	RECURRENCE			
11	BILATERAL			
12	SURGERY	1	2	3
	RADIO THERAPY (DATES)			
	CHEMOTHERAPY (TYPE)			
	HORMONE (TYPE)			
13	OTHER CANCER	TYPE	DATE	PREVIOUS SUBSEQUENT
14	OTHER CANCER TREATMENT			
	SURGERY			
	RADIO THERAPY (DATES)			
	CHEMOTHERAPY (TYPE)			
	HORMONE (TYPE)			
15	METASTSES	SITE(S)	DATE(S)	DIAGNOSED
16	LAST OPA	DATE	HOSPITAL	
	NON-HOSP FOLLOW UP INFO.		DATE	FROM
17	DEATH DETAILS	DATE	REG. CAUSE OF DEATH	
18	COMMENTS (any other relevant information)			

A

-----,MISS-----

Date of first report 6.2.81

GENDER : sex : female

PATHOLOGY

6.2.81 Report : Pathology Number = --/-- ; side : right; DCIS; foci : not stated; Comment = papillary intraduct ca.

2.12.81 Report : Pathology Number = --/-- ; side : right; Recurrence; DCIS foci : not stated

21.8.85 Report : Pathology Number = --/-- ; side : right; Recurrence; DCIS Invasive; foci : not stated

Reviewed : 18.9.92 Path Number(s) selected = --/--; --/-- BLOCKS 1 2

SLIDES : 8.9.93 slides cut

LAST UPDATE (SLIDE AND BLOCK LOCATION) : 19.8.92 Comment = 1985: 2999/85
intraduct and infiltrating papillary
ca. Slides not in FR Path. - out to
B.G.; slides location : AW; block
location : FR
4.9.92 slides location : BG; block
location : FR
18.9.92 slides location : FR; block
location : FR

-----,MISS-----

Date of first report 6.2.81

Family History : Family History of Breast Cancer; Mother; Age at diagnosis
= NK

Pathology Details : Date of Birth = 6.2.24; Pathology reference number(s) =
--/81; Date of Report = 6.2.81; Slides reviewed; Slides
selected; Symptom status : symptomatic; side : Right; site
: Other; specify = CENTRAL; Histological calcification :
NK; Specimen type : open biopsy; Specimen size (excl
mastectomy & needle core biopsy) : known; Largest diameter
(mm) = 65; Second largest diameter (mm) = 30; Smallest
diameter (mm) = 15; Size of second specimen : Not
applicable; Histological diagnosis : Abnormal; malignant;
Epithelial Proliferation : not present; malignant type :
non-invasive; ductal; cribriform; Papillary;
micropapillary; microinvasion : not present; Axillary
nodes : not present; Other nodes : not present; Excision :
NK; Grade : not assessable; disease extent : diffuse
single quadrant; vascular invasion : NK

BILATERAL CASES

TOTAL NUMBER OF PATIENTS WITH IN-SITU CARCINOMA OF BREAST
IDENTIFIED TO DATE: 501

TOTAL PATIENTS WITH BILATERAL CA. BREAST: 73

(BILATERAL includes:

- 1 bilateral in-situ carcinoma of the breast
- 2 in-situ carcinoma in one breast with invasive carcinoma
in the contralateral breast)

SYNCHRONOUS	METACHRONOUS	TOTAL
31	42	73